

We claim:

1. A recombinant  $\text{Ca}^{2+}$  dependent monoclonal antibody immunoreactive with an epitope in the activation peptide region of the heavy chain of Protein C defined by E D Q V D P R L I D G K (Sequence ID No. 1) in combination with calcium, where the antibody inhibits Protein C activation by thrombin-thrombomodulin.

2. The antibody of claim 1 comprising amino acid sequence selected from the group consisting of:

MGR LSSS FLL LIAPAYVLSQ VTLKESGPGI LQPSQTLT LT CSLSGFSLRT  
SGMGVGWIRQ PSGKGLEWLA HIWDDDKRY NPVLKSRLII SKDTSRKQVF  
LKIASVDTAD TATYYCVRMM DDYDAMDYWG QGTSVTVSS (Sequence  
ID No. 10); MDFQVQIFSF LLISASVIMS RGQIILTQSP  
AIMSASLGEE ITLTCSATSS VTYVHWYQQK SGTSPKLLIY GTSNLAGVGP  
SRFSGSGSGT FYSLTVSSVE AEDAADYYCH QWNSYPHTFG GGTKLEIKR  
(Sequence ID No. 12); Q VTLKESGPGI LQPSQTLT LT  
CSLSGFSLRT SGMGVGWIRQ PSGKGLEWLA HIWDDDKRY NPVLKSRLII  
SKDTSRKQVF LKIASVDTAD TATYYCVRMM DDYDAMDYWG QGTSVTVSS  
(amino acids 20-139 of Sequence ID No. 10) and  
QIILTQSP AIMSASLGEE ITLTCSATSS VTYVHWYQQK SGTSPKLLIY  
GTSNLAGVGP SRFSGSGSGT FYSLTVSSVE AEDAADYYCH QWNSYPHTFG  
GGTKLEIKR (amino acids 23-129 of Sequence ID No. 12).

3. The antibody of claim 1 containing human amino acid sequence.

4. The antibody of claim 1 encoded in part by a nucleotide sequence selected from the group consisting of ATGGGCAGGC TTTCTTCTTC ATTCTTGCTA  
CTGATTGCCC CTGCATATGT CCGTCCAG GTTACTCTGA AAGAGTCTGG  
CCCTGGGATA TTGCAGCCCT CCGAGACCCT CACTCTGACT TGTCTCTCT  
CTGGGTTTTC ACTGAGGACT TCTGGTATGG GTGTAGGCTG GATTCGTCAG  
CCTTCAGGGA AGGGTCTGGA GTGGCTGGCA CACATTTGGT GGGATGATGA  
CAAGCGCTAT AACCCAGTCC TGAAGAGCCG ACTGATAATC TCCAAGGATA

Sub  
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HPC4 V<sub>H</sub>  
(P17)

HPC4  
V<sub>L</sub>

CCTCCAGGAA ACAGGTATTC CTCAAGATCG CCAGTGTGGA CACTGCAGAT  
 ACTGCCACAT ACTACTGTGT TCGAATGATG GATGATTACG ACGCTATGGA  
 CTACTGGGGT CAAGGAACCT CAGTCACCGT CTCCTCT (Sequence ID  
 No. 9); CAG GTTACTCTGA AAGAGTCTGG CCCTGGGATA  
 TTGCAGCCCT CCCAGACCCT CACTCTGACT TGTTCCTCTCT CTGGGTTTTC  
 ACTGAGGACT TCTGGTATGG GTGTAGGCTG GATTCGTCAG CCTTCAGGGA  
 AGGGTCTGGA GTGGCTGGCA CACATTTGGT GGGATGATGA CAAGCGCTAT  
 AACCCAGTCC TGAAGAGCCG ACTGATAATC TCCAAGGATA CCTCCAGGAA  
 ACAGGTATTC CTCAAGATCG CCAGTGTGGA CACTGCAGAT ACTGCCACAT  
 ACTACTGTGT TCGAATGATG GATGATTACG ACGCTATGGA CTACTGGGGT  
 CAAGGAACCT CAGTCACCGT CTCCTCT (nucleotides 58 to 417  
 of Sequence ID No. 9); ATGGATTTTC AGGTGCAGAT  
 TTTCAGCTTC CTGCTAATCA GTGCCTCAGT CATAATGTCC AGAGGACAAA  
 TTATTCTCAC CCAGTCTCCG GCAATCATGT CTGCATCTCT GGGGGAGGAG  
 ATCACCTAA CCTGCAGTGC CACTTCGAGT GTAACCTACG TCCACTGGTA  
 CCAGCAGAAG TCAGGCACTT CTCCCAAACCT CTTGATTTAT GGGACATCCA  
 ACCTGGCTTC TGGAGTCCCT TCTCGTTTCA GTGGCAGTGG  
 GTCTGGGACC TTTTATTCTC TCACAGTCAG CAGTGTGGAG GCTGAAGATG  
 CTGCCGATTA TTAAGTCCAT CAGTGGGAATA GTTATCCGCA CACGTTCCGA  
 GGGGGGACCA AGCTGGAAAT AAAACGG (Sequence ID No. 11); - HPC-4 VL  
 CAAA TTATTCTCAC CCAGTCTCCG GCAATCATGT CTGCATCTCT  
 GGGGGAGGAG ATCACCTAA CCTGCAGTGC CACTTCGAGT GTAACCTACG  
 TCCACTGGTA CCAGCAGAAG TCAGGCACTT CTCCCAAACCT CTTGATTTAT  
 GGGACATCCA ACCTGGCTTC TGGAGTCCCT TCTCGTTTCA  
 GTGGCAGTGG GTCTGGGACC TTTTATTCTC TCACAGTCAG CAGTGTGGAG  
 GCTGAAGATG CTGCCGATTA TTAAGTCCAT CAGTGGGAATA GTTATCCGCA  
 CACGTTCCGA GGGGGGACCA AGCTGGAAAT AAAACGG (nucleotides  
 67 to 387 of Sequence ID No. 11); and degenerate  
 sequences thereof.

5. The antibody of claim 1 further  
 comprising a pharmaceutically acceptable carrier for  
 administration to a patient.

6. The antibody of claim 5 further  
 comprising a cytokine or an inducer of cytokine  
 expression in a dosage effective in combination with

the antibody to coagulate microvasculature in tumors but not in the absence of the antibody.

7. The antibody of claim 1 having a detectable label bound to the antibody.

8. The antibody of claim 1 immobilized to a substrate, wherein the immobilized antibody is suitable for purification of protein C from a biological fluid.

9. A method for treating a disorder by inhibition of protein C anticoagulant comprising administering to a patient in need of treatment thereof an effective amount of a recombinant  $Ca^{2+}$  dependent monoclonal antibody immunoreactive with an epitope in the activation peptide region of the heavy chain of Protein C defined by E D Q Y D P R L I D G K (Sequence ID No. 1) in combination with calcium, where the antibody inhibits Protein C activation by thrombin-thrombomodulin.

10. The method of claim 9 wherein the antibody comprises amino acid sequence selected from the group consisting of:

MGR LSSS FLL LIAPAYVLSQ VTLKESGPGI LQPSQTLTLT CSLSGFSLRT  
SGMGVGWIRQ PSGKGLEWLA HIWDDDKRY NPVLKSRLII SKDTSRKQVF  
LKIASVDTAD TATYYCVRMM DDYDAMDYWG QGTSVTVSS (Sequence  
ID No. 10); MDFQVQIFSF LLISASVIMS RGQIILTQSP  
AIMSASLGEE ITLTCSATSS VTYVHWYQQK SGTSPKLLIY GTSNLAGVGP  
SRFSGSGSGT FYSLTVSSVE AEDAADYYCH QWNSYPHTFG GGTKLEIKR  
(Sequence ID No. 12); Q VTLKESGPGI LQPSQTLTLT  
CSLSGFSLRT SGMGVGWIRQ PSGKGLEWLA HIWDDDKRY NPVLKSRLII  
SKDTSRKQVF LKIASVDTAD TATYYCVRMM DDYDAMDYWG QGTSVTVSS  
(amino acids 20-139 of Sequence ID No. 10) and  
QIILTQSP AIMSASLGEE ITLTCSATSS VTYVHWYQQK SGTSPKLLIY  
GTSNLAGVGP SRFSGSGSGT FYSLTVSSVE AEDAADYYCH QWNSYPHTFG  
GGTKLEIKR (amino acids 23-129 of Sequence ID No. 12).

11. The method of claim 9 wherein the antibody contains human amino acid sequence.

12. The method of claim 9 wherein the antibody is encoded in part by a nucleotide sequence selected from the group consisting of ATGGGCAGGC

TTTCTTCTTC ATTCTTGCTA CTGATTGCCC CTGCATATGT CCTGTCCCAG  
 GTTACTCTGA AAGAGTCTGG CCCTGGGATA TTGCAGCCCT CCCAGACCCT  
 CACTCTGACT TGTTCTCTCT CTGGGTTTTC ACTGAGGACT TCTGGTATGG  
 GTGTAGGCTG GATTCGTCAG CCTTCAGGGA AGGCTCTGGA GTGGCTGGCA  
 CACATTTGGT GGGATGATGA CAAGCGCTAT AACCCAGTCC TGAAGAGCCG  
 ACTGATAATC TCCAAGGATA CCTCCAGGAA ACAGGTATTC CTCAAGATCG  
 CCAGTGTGGA CACTGCAGAT ACTGCCACAT ACTACTGTGT TCGAATGATG  
 GATGATTACG ACGCTATGGA CTA CTGGGGT CAAGGAACCT CAGTCACCGT  
 CTCCTCT (Sequence ID No. 9); CAG GTTACTCTGA AAGAGTCTGG  
 CCCTGGGATA TTGCAGCCCT CCCAGACCCT CACTCTGACT TGTTCTCTCT  
 CTGGGTTTTC ACTGAGGACT TCTGGTATGG GTGTAGGCTG GATTCGTCAG  
 CCTTCAGGGA AGGCTCTGGA GTGGCTGGCA CACATTTGGT GGGATGATGA  
 CAAGCGCTAT AACCCAGTCC TGAAGAGCCG ACTGATAATC TCCAAGGATA  
 CCTCCAGGAA ACAGGTATTC CTCAAGATCG CCAGTGTGGA CACTGCAGAT  
 ACTGCCACAT ACTACTGTGT TCGAATGATG GATGATTACG ACGCTATGGA  
 CTA CTGGGGT CAAGGAACCT CAGTCACCGT CTCCTCT (nucleotides  
 58 to 417 of Sequence ID No. 9); ATGGATTTTC AGGTGCAGAT  
 TTTCAGCTTC CTGCTAATCA GTGCCTCAGT CATAATGTCC AGAGGACAAA  
 TTATTCTCAC CCAGTCTCCG GCAATCATGT CTGCATCTCT GGGGGAGGAG  
 ATCACCTAA CCTGCAGTGC CACTTCGAGT GTAACCTACG TCCACTGGTA  
 CCAGCAGAAG TCAGGCACTT CTCCCAAAT CTTGATTTAT GGGACATCCA  
 ACCTGGCTTC TGGAGTCCCT TCTCGTTTCA GTGGCAGTGG  
 GTCTGGGACC TTTTATTCTC TCACAGTCAG CAGTGTGGAG GCTGAAGATG  
 CTGCCGATTA TTA CTGCCAT CAGTGGAATA GTTATCCGCA CACGTTCCGA  
 GGGGGGACCA AGCTGGAAAT AAAACGG (Sequence ID No. 11);  
 CAAA TTATTCTCAC CCAGTCTCCG GCAATCATGT CTGCATCTCT  
 GGGGGAGGAG ATCACCTAA CCTGCAGTGC CACTTCGAGT GTAACCTACG  
 TCCACTGGTA CCAGCAGAAG TCAGGCACTT CTCCCAAAT CTTGATTTAT  
 GGGACATCCA ACCTGGCTTC TGGAGTCCCT TCTCGTTTCA  
 GTGGCAGTGG GTCTGGGACC TTTTATTCTC TCACAGTCAG CAGTGTGGAG

GCTGAAGATG CTGCCGATTA TTACTGCCAT CAGTGAATA GTTATCCGCA  
CACGTTCCGA GGGGGGACCA AGCTGGAAAT AAAACGG (nucleotides  
67 to 387 of Sequence ID No. 11); and degenerate  
sequences thereof.

13. The method of claim 9 further  
comprising administering with the antibody a cytokine  
or other chemotherapeutic agent in an amount effective  
to coagulate the microvasculature of a tumor.

14. A method of making a recombinant  $\text{Ca}^{2+}$   
dependent monoclonal antibody immunoreactive with an  
epitope in the activation peptide region of the heavy  
chain of Protein C defined by E D Q V D P R L I D G K  
(Sequence ID No. 1) in combination with calcium, where  
the antibody inhibits Protein C activation by  
thrombin-thrombomodulin, by expressing nucleotide  
sequence encoding the antibody.

15. The method of claim 14 wherein the  
antibody comprises amino acid sequence selected from  
the group consisting of:  
MGRLLSSSFL LIAPAYVLSQ VTLKESGPGI LQPSQTLTLT CSLSGFSLRT  
SGMGVGWIRQ PSGKGLEWLA HIWDDDKRY NPVLKSRLII SKDTSRKQVF  
LKIASVDTAD TATYYCVRMM DDYDAMDYWG QGTSVTVSS (Sequence  
ID No. 10); MDFQVQIFSF LLISASVIMS RGQIILTQSP  
AIMSASLGEE ITLTCSATSS VTYVHWYQQK SGTSPKLLIY GTSNLAGVP  
SRFSGSGSGT FYSLTVSSVE AEDAADYYCH QWNSYPHTFG GGTKLEIKR  
(Sequence ID No. 12); Q VTLKESGPGI LQPSQTLTLT  
CSLSGFSLRT SGMGVGWIRQ PSGKGLEWLA HIWDDDKRY NPVLKSRLII  
SKDTSRKQVF LKIASVDTAD TATYYCVRMM DDYDAMDYWG QGTSVTVSS  
(amino acids 20-139 of Sequence ID No. 10) and  
QIILTQSP AIMSASLGEE ITLTCSATSS VTYVHWYQQK SGTSPKLLIY  
GTSNLAGVP SRFSGSGSGT FYSLTVSSVE AEDAADYYCH QWNSYPHTFG  
GGTKLEIKR (amino acids 23-129 of Sequence ID No. 12).

16. The method of claim 14 wherein the  
antibody is encoded in part by a nucleotide sequence

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selected from the group consisting of ATGGGCAGGC  
 TTTCTTCTTC ATTCTTGCTA CTGATTGCCC CTGCATATGT CCTGTCCCAG  
 GTTACTCTGA AAGAGTCTGG CCCTGGGATA TTGCAGCCCT CCCAGACCCT  
 CACTCTGACT TGTTCTCTCT CTGGGTTTTTC ACTGAGGACT TCTGGTATGG  
 GTGTAGGCTG GATTCGTCAG CCTTCAGGGA AGGGTCTGGA GTGGCTGGCA  
 CACATTTGGT GGGATGATGA CAAGCGCTAT AACCCAGTCC TGAAGAGCCG  
 ACTGATAATC TCCAAGGATA CCTCCAGGAA ACAGGTATTC CTCAAGATCG  
 CCAGTGTGGA CACTGCAGAT ACTGCCACAT ACTACTGTGT TCGAATGATG  
 GATGATTACG ACGCTATGGA CTACTGGGGT CAAGGAACCT CAGTCACCGT  
 CTCCTCT (Sequence ID No. 9); CAG GTTACTCTGA AAGAGTCTGG  
 CCCTGGGATA TTGCAGCCCT CCCAGACCCT CACTCTGACT TGTTCTCTCT  
 CTGGGTTTTTC ACTGAGGACT TCTGGTATGG GTGTAGGCTG GATTCGTCAG  
 CCTTCAGGGA AGGGTCTGGA GTGGCTGGCA CACATTTGGT GGGATGATGA  
 CAAGCGCTAT AACCCAGTCC TGAAGAGCCG ACTGATAATC TCCAAGGATA  
 CCTCCAGGAA ACAGGTATTC CTCAAGATCG CCAGTGTGGA CACTGCAGAT  
 ACTGCCACAT ACTACTGTGT TCGAATGATG GATGATTACG ACGCTATGGA  
 CTACTGGGGT CAAGGAACCT CAGTCACCGT CTCCTCT (nucleotides  
 58 to 417 of Sequence ID No. 9); ATGGATTTTC AGGTGCAGAT  
 TTTCAGCTTC CTGCTAATCA GTGCCTCAGT CATAATGTCC AGAGGACAAA  
 TTATTCTCAC CCAGTCTCCG GCAATCATGT CTGCATCTCT GGGGGAGGAG  
 ATCACCCTAA CCTGCAGTGC CACTTCGAGT GTAACCTACG TCCACTGGTA  
 CCAGCAGAAG TCAGGCACTT CTCCCAAACCT CTTGATTTAT GGGACATCCA  
 ACCTGGCTTC TGGAGTCCCT TCTCGTTTCA GTGGCAGTGG  
 GTCTGGGACC TTTTATTCTC TCACAGTCAG CAGTGTGGAG GCTGAAGATG  
 CTGCCGATTA TTAAGTCCAT CAGTGAATA GTTATCCGCA CACGTTCCGA  
 GGGGGGACCA AGCTGGAAAT AAAACGG (Sequence ID No. 11);  
 CAAA TTATTCTCAC CCAGTCTCCG GCAATCATGT CTGCATCTCT  
 GGGGGAGGAG ATCACCCTAA CCTGCAGTGC CACTTCGAGT GTAACCTACG  
 TCCACTGGTA CCAGCAGAAG TCAGGCACTT CTCCCAAACCT CTTGATTTAT  
 GGGACATCCA ACCTGGCTTC TGGAGTCCCT TCTCGTTTCA  
 GTGGCAGTGG GTCTGGGACC TTTTATTCTC TCACAGTCAG CAGTGTGGAG  
 GCTGAAGATG CTGCCGATTA TTAAGTCCAT CAGTGAATA GTTATCCGCA  
 CACGTTCCGA GGGGGGACCA AGCTGGAAAT AAAACGG (nucleotides  
 67 to 387 of Sequence ID No. 11); and degenerate  
 sequences thereof.

Sub  
A5 7 17. The method of claim 14 further  
comprising inserting human sequence into the antibody  
in place of animal sequence.

Sub  
C5 7 18. The method of claim 14 further  
comprising binding detectable label to the antibody.

Sub  
A6 7 19. The method of claim 14 further  
comprising immobilizing the antibody to a substrate,  
wherein the immobilized antibody is suitable for  
purification of protein C from a biological fluid.

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